STUDIES ON THE GROWTH OF INTEGUMENTARY PIGMENT IN THE LOWER VERTEBRATES

II. The Rôle of the Hypophysis in Melanogenesis in the Common Catfish (Ameiurus melas) [1]

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In previous communications (Osborn, 1940*a*, *b*, and *c*) it was shown that smooth-skinned as well as scaly teleosts responded to experimental procedures by developing melanophores on their normally unpigmented surface. These findings confirm and extend the earlier works of Cunningham (1891, 1893); von Frisch (1911); Herbst and Ascher (1927) and others more completely listed in another paper (Osborn, 1941, in press).

In several experiments concerned with the experimental growth of melanophores it has been observed consistently that these cells developed most rapidly and abundantly when the fish was in a physiological condition which caused pigment granules in the normally existing melanophores to be maximally dispersed (Osborn, 1940*a* and *b* and 1941 in press). Odiorne (1937) suggested that "Any condition leading to the dispersion of pigment throughout the cell will, if maintained, promote the development of melanophores or insure their continued existence"; and further wrote that "The neurohumors which are instrumental in bringing about the pigmentary migrations in Fundulus also exert trophic influences upon the melanophores."

In the catfish it has been found that melanophores can be grown experimentally on the naturally white belly of the animal by directing light upon it while the pigmented dorsal surface is in the dark phase (Osborn, 1940*c*). If the fish is white-adapted, however, ventral illumination does not result in melanophore formation. It was previously shown (Osborn, 1938) that the melanophore-dispersing principle of the hypophysis plays a major rôle in producing the dark phase (physiological darkening) in the natural color changes of the animal. By removing the source of this secretion but maintaining constant all other conditions favoring the growth of melanophores on the white belly surface, it

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should be possible to determine whether a substance necessary in physiological darkening was also essential in the production of melanophores (morphological darkening). In the experiments to be reported here the fact that melanophores failed to grow experimentally in hypophysectomized catfishes indicates that a substance necessary to produce the dark phase is also indispensable to the experimental development of melanophores. This strongly suggests that morphological color change is not the result of physiological color change but rather that both are the product of a common underlying mechanism which effects the former change more slowly than the latter.

Materials and Methods

Common catfishes (*Ameiurus melas*) six to eight inches in length were kindly furnished me by Dr. T. H. Langlois, director of the Franz Theodore Stone Laboratory, Put-In-Bay, Ohio.[2] The laboratory stock was kept in muddy water at 12 to 18° C. in large gray tanks in an animal room where the illumination was of low intensity and darkness was maintained at night. Under such circumstances fishes have been kept over a year in excellent condition and have maintained normal pigmentation. The experimental fishes were kept in water at 10 to 12° C. during the first post-operative week and henceforth the temperature was maintained between 14 and 18° C. At this temperature they took food regularly: rolled oats daily and bits of liver or ground beef about once a week.

Illumination was directed to the ventral surface of the experimental fishes either by specially constructed glass-bottomed tubs with ceiling and sides black or white (Osborn, 1940*a*) or by reflection from white tubs brightly illuminated from above. Both of these light sources have been used successfully in growing ventral melanophores. In our apparatus illumination by reflection grows pigment less rapidly, however, because of the lower intensity of the light actually falling upon the lower surface of the fish.

The fishes, after having been lightly anesthetized in a dilute chloretone solution or stupefied by chilling, were totally blinded by enucleation and were hypophysectomized by the oral approach. Hypophysectomies were checked for completeness by reconstructions at the time of operating, by observing the post-operative color changes displayed by each fish and by examination at autopsy. When for any reason the operation was considered imperfect the data for that animal were discarded.

Some of the fishes were sacrificed at convenient intervals for microscopic study, others for chemical determinations. In almost all cases

[2] Courtesy of Mr. John Sullivan, Ohio Conservation Department.

TABLE I

	Animals alive 30 days after beginning of experiment	
	Number	Percentage of original
Group A	43	71.7
Group B	29	97.0
Group C	16	72.7
Group D	15	100.0

photographs were taken of living fishes but in certain instances additional records of preserved animals were made.

EXPERIMENTAL

In these experiments over a hundred catfishes were used representing four different physiological or operative conditions as follows:

Group A—60 fishes—totally blinded; hypophysectomized 12 hrs. later.
Group B—30 fishes—totally blinded.
Group C—22 fishes—hypophysectomized only.
Group D—15 fishes—unoperated controls.

Animals from each of the above groups were placed in each of five experimental tubs: four providing continuous direct ventral illumination (apparatus only slightly modified from that previously described, Osborn 1940*a*) and one having a white bottom which reflected light to the belly of the fishes. By having representative fishes from each of the groups in every tub, any possible effects of slight differences in temperature, light intensity, feeding, etc. were automatically ruled out. The

PLATE I

EXPLANATION OF FIGURES

Figures 1 and 2 are ventral views of two fishes described below (about ⅔ natural size). Figures 3 and 4 are lateral views of the same two fishes.

FIGS. 1 AND 3. A common catfish (*Ameiurus melas*) blinded and continuously illuminated ventrally by light reflected from the white bottom of the tub in which this experimental fish was kept for 125 days. Note dark shade and excessive ventral melanination.

FIGS. 2 AND 4. A catfish blinded, 12 hours later hypophysectomized and maintained 125 days in the white tub described above continuously illuminated. Note the pale shade and relative loss of pigment compared with blinded control Fig. 8. The fishes in Figs. 1 and 2; 3 and 4 were photographed and printed together.

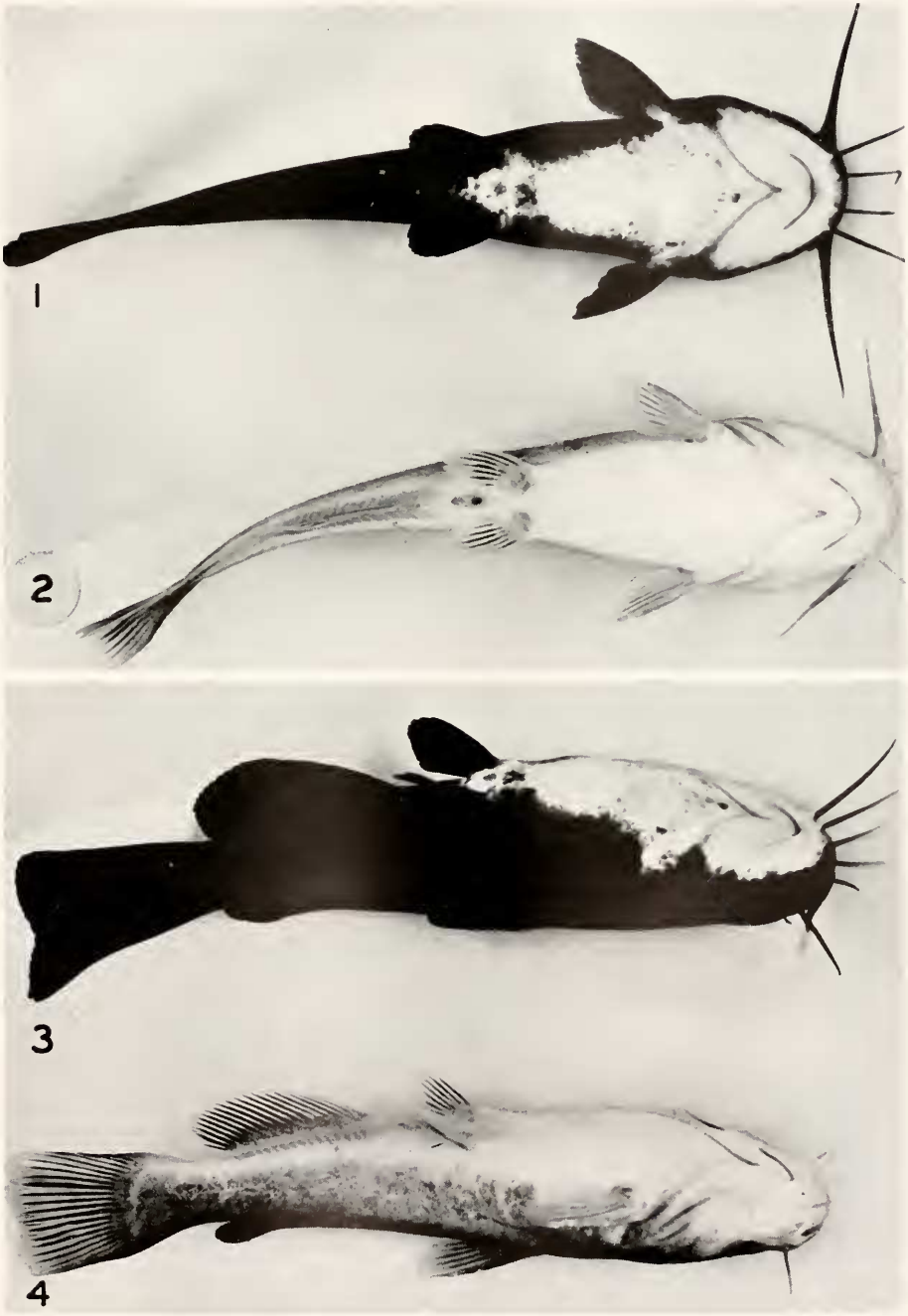


PLATE I

animals were checked twice daily for mortalities and dead or dying fishes were placed in fixative immediately and observations recorded.

Because at least a month of continuous illumination (at the intensity used) was required to develop more than a slight amount of ventral pigment in the catfish, data on fishes surviving less than 30 days were discarded. It is important, therefore, to list animals surviving the operation by 30 days. About 80 per cent of the original fishes were alive and distributed as shown in Table I.

After the first month the mortality rate decreased, presumably because the less vigorous animals succumbed earliest. After that, occasional deaths, combined with the intentional sacrifice of an animal now and then, reduced the number of experimental fishes considerably so that at the end of 180 days 51 animals (about 40 per cent) surviving in good condition were distributed in the four groups as shown in Table II.

TABLE II

	Animals alive at end of experiment—180 days	
	Number	Percentage of original
Group A	11	18.3
Group B	23	76.7
Group C	6	27.3
Group D	11	73.3

At the end of the experiment (180 days) representative animals from each group were reserved for chemical and other quantitative

PLATE II

EXPLANATION OF FIGURES

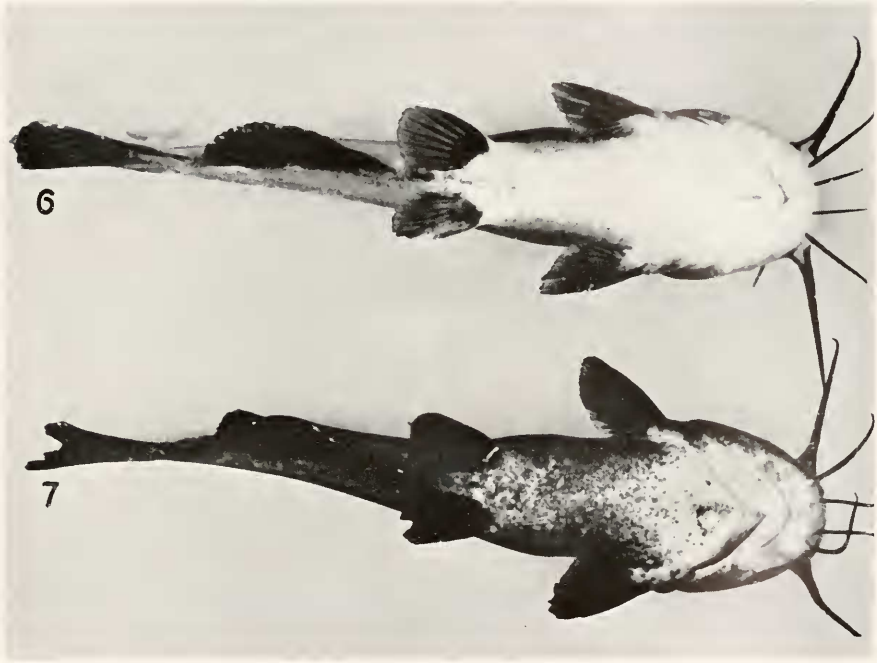
All ventral views—about ⅔ natural size.

FIG. 5. A catfish blinded and subjected to *direct* ventral illumination for 148 days. Note how the normally unpigmented white vest has become almost completely blackened with melanophores. Direct illumination grows the pigment faster than weaker reflected light. Compare with Fig. 1.

FIG. 6. A catfish (eyes intact) ventrally illuminated with direct light for 55 days. The animal remained somewhat dark-adapted to the black sides and ceiling of the tub. Note that some ventral pigment has grown, especially at the base of the anal fin. Compare with Fig. 8.

FIG. 7. A catfish blinded and ventrally illuminated (direct light) 79 days. The pigmentation is somewhat less extensive than in Fig. 5.

FIG. 8. An animal blinded and kept with the stock fishes in an unlighted tank of neutral shade 76 days. Note the dark shade resulting from blinding, but excessive pigmentation has not occurred. This fish serves as an appropriate control for some of the other animals illustrated.



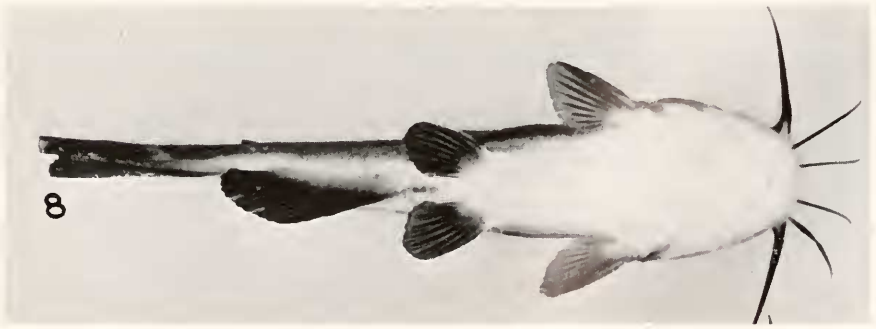


PLATE II

determinations, the results of which will be assembled in a later communication. Only qualitative results will be reported here.

Results

After prolonged treatment under the conditions described, the animals from all four experimental groups could be divided into only two categories on the basis of general macroscopic appearance.

On the one hand fishes of group B which had been blinded only (hypophysis intact) had become coal black dorsally and laterally and had developed dense ventral pigment especially around the cloacal aperture and posteriorly around the anal fin in addition to numerous spots of melanophores scattered over the normally white vest of the belly surface (Figs. 1, 3, 5 and 7). The deposition of melanin in these fishes was strikingly excessive as evidenced by the amount of black pigment which came off on one's hands when the fishes were handled for observations.[3] This never occurred in handling fishes of any of the three other experimental groups.

On the other hand, fishes of groups A (blinded and hypophysectomized), C (hypophysectomized—eyes intact) and D (unoperated) were all *pale in shade* (except those in group D in black-walled tubs) and in general not easily distinguished by original groups although in some instances those in group A seemed slightly darker than fishes in groups C and D. Whether this was a significant difference may be decided from future quantitative determinations. Of interest in our present findings is the fact that hypophysectomized animals of group A were unable to grow melanophores (Figs. 2 and 4) while those in group B, alike in all experimental details except that the hypophysis was functionally intact, grew abundant melanophores. All fishes in both groups had been totally blinded, an operation which in normal catfishes results in the pronounced darkening of the integument (Fig. 8) due to maximal dispersion of the melanin granules within the melanophores (Parker, 1934 and 1939; Abramowitz, 1936; Osborn, 1938). When animals thus blinded are hypophysectomized, however, the integument pales considerably with corresponding concentration of the melanin granules (Osborn, 1938). It appears, then, that when the pigmentary system is subjected to these two opposing influences, melanogenesis is not accelerated even though the external environment (illumination, etc.) strongly favors the growth of melanophores. Furthermore, the melanophores are not maintained normally but rather undergo gradual degeneration.

[3] This is in all probability the result of large numbers of superficial melanophores being cast off through the epidermis, a condition invariably found in catfish integuments where melanin production is going on at an accelerated rate.

Animals in groups C and D placed in white tubs remained very pale and no evidence of accelerated melanogenesis was observed although ventral illumination was continuously provided. Animals in group D were actually white-adapted normal animals (tub walls and ceiling white) while those in group C not only were white-adapted but were deprived of the hypophysis, the source of the chief melanin-dispersing factor in the normal chromatology of the catfish. Of the other fishes in groups C and D in tubs with black walls and ceiling, those in group C were very slightly darker qualitatively than corresponding fishes in white tubs while group D catfishes were rather black-adapted with a noticeable increase in pigmentation (Fig. 6).

It was noticed that the animals of groups A, C and some in D (those in white tubs) not only failed to show evidence of accelerated melanogenesis but actually appeared less heavily pigmented at the end of the experiment than stock controls.

Discussion

Two types of color change have been recognized for several years, rapid and gradual. The thesis that there is a causal relation between the phenomena of transitory and of quantitative color change referred to as "Babak's Law" recognizes as separate features the rapid color changes and those of a very gradual, less temporary type. Odiorne (1937) speaks of these as "physiological" and "morphological" color changes respectively. He found that the pigmentation of *Fundulus majalis, F. heteroclitus,* and *Ameiurus nebulosus* is "reduced through the degeneration of melanophores when these fishes are kept on white backgrounds, but tends to increase when they are kept upon black backgrounds." He also reported that "The development of pigmentation in young fishes (*Macropodus* and *Gambusia*) is retarded if they are kept on white backgrounds, but on black backgrounds the fishes become very dark." Odiorne concluded that "Morphological color changes (alterations in pigmentation) and physiological color changes (arising from pigmentary movements) are phenomena resulting from a common cause."

Other investigators (von Frisch, 1911; Vilter, 1931; Sumner and Wells, 1933; Sumner and Doudoroff, 1937; Sumner, 1939 and 1940*a* and *b*; and Dawes, 1941) have reported experiments concerning an increase or decrease in integumentary melanin. So far as the writer is aware, every case of melanin increase was associated with a condition favoring melanin dispersion in the cells, whereas decreases in melanin regularly occurred in animals maintained in the pale phase over extended

periods. These observations are in total agreement with the conclusions of Odiorne, but direct evidence to indicate that a substance active in physiological color change is also necessary for the formation of new melanophores has hitherto been lacking. The results recorded here indicate that the melanophore-dispersing substance of the pituitary gland, so important in producing the dark phase of the catfish in its normal physiology (Osborn, 1938), is also necessary for the development of new integumentary melanophores and for the maintenance of those already formed. When this substance is absent from the blood (in hypophysectomized fishes), new melanophores are not developed even when otherwise optimum conditions for their growth are maintained. This is most clearly seen in the white normally non-melaninated vest of the fish, which will become pigmented with melanophores under the conditions described in group B (Figs. 1, 3, 5 and 7), using ventral illumination. Not only did such pigmentation fail to occur in catfishes whose pituitaries had been removed, but many of the melanophores present previous to the operation underwent degeneration.

These findings suggest that the melanophore-dispersing substance circulated in the blood stream of the normal fish provides a favorable medium (internal environment) in which melanogenesis may go on. We do not yet know, of course, whether this pituitary fraction itself enters actively into the chemistry of melanin formation or whether it acts as a catalyst in some way. In this connection it is of interest to note that Fostvedt (1940) has reported that some pituitary fractions especially high in melanophore-hormone content produced marked acceleration of the oxidase system in the tyrosine-tyrosinase reaction. This was shown in hypophysectomized frogs whose legs, isolated, were incubated for specified periods of time following injection with the extract. Although this is somewhat removed from catfish chromatology it suggests, at least, how the melanophore hormone may enter into melanin formation naturally, especially in animals whose normal color change mechanism is so highly dependent upon this pituitary secretion.

Incidental to other observations, Rahn (1941) noticed in the rattlesnake that following hypophysectomy little, if any, melanin was deposited into the cells of the shedding stratum corneum. This probably indicates a failure of the melanophores to produce normal amounts of pigment in the absence of the hypophysis. Recent clinical reports by Fournier, Cervino and Conti (1941) indicate success with local injections of melanophore hormone in the treatment of vitiligo in man. Their illustrations show clearly that pigment-free patches become repigmented under administration of the hormone. This finding, together with earlier reports by With (1920), Buschke (1907) and others who

treated vitiligo successfully by stimulating the growth of pigment with light baths indicates that in the human being dual factors (*light* externally and a *hormone* internally) may coöperate in the growth and maintenance of pigment. It is interesting that similar agents are shown here to control pigment production and maintenance in a teleost.

Because facts in this field are just beginning to accumulate, anything more than speculation would be quite premature. Is it not conceivable, however, that the intermedin abundant in the mammalian hypophysis might be concerned in maintaining the degree of pigmentation peculiar to the individual and that an imbalance of this factor might be correlated with certain pathologies where active melanogenesis is characteristic?

Summary

The common catfish (*Ameiurus melas*) possesses naturally a white vest ventrally in which melanophores are only rarely found. In appropriate apparatus it is possible to grow melanophores abundantly over this naturally unpigmented area and increase the amount of pigment in other areas if the dorsal aspect (normally pigmented surfaces) of the fish is maintained in the dark phase. It is convenient, though not necessary, to continue the dark phase permanently by blinding the fish totally, a fact which "per se" indicates that the eyes are not necessary in active melanogenesis.

If the pituitary gland is removed, however, melanogenesis does not continue. In fact, melanophore degeneration sets in with the end result that the experimental fish is paler and less heavily melaninated than stock controls. This indicates that the melanophore-dispersing hormone of the pituitary gland so important in the normal color change physiology of the catfish is also indispensable to the development and maintenance of melanin in melanophores. Interpreted in another way, it suggests that morphological color change is not produced by physiological color change but rather that both are the result of a common underlying mechanism.

A possible way in which the melanophore-dispersing fraction of the pituitary may be involved in the production of melanin is discussed. It is suggested that the melanophore-dispersing hormone (intermedin) in the human hypophysis may be concerned in the production and maintenance of normal pigmentation in man.

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